THE RELATIONSHIP BETWEEN THE PITUITARY GLAND AND THE GONADS IN FUNDULUS

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Introduction

From the extensive work on mammals, birds, reptiles and amphibians it is clear that in these forms the anterior lobe of the pituitary gland is an essential factor in the control of the sex cycle. In teleost fishes, however, there is little evidence concerning the rôle of the pituitary body. Houssay (1930, 1931) found that the injection of saline suspensions of the hypophyses from large fish (*Micropogon opercularis*) into small ones (*Cnesterodon*) was followed by the expulsion of eggs in from one to three days, although saline suspensions of muscle or saline solution alone produced no effect in control animals. This expulsion of eggs, however, preceded normal spawning by only 15 days. Cardoso (1934) injected saline suspensions of the pituitary bodies of *Pimelodus clarias* into other individuals of the same species. In both sexes he noted an increase in weight of the gonads over those of control animals, an effect much greater in the female. Pereira and Cardoso (1934), working with *Prochilodus,* injected saline suspensions of pituitary glands of the adult into females. In all cases ovulation occurred, varying from 24 to 96 hours after injection, although the injection of suspensions of nervous tissue into control animals did not result in ovulation. More recently von Ihering (1935) repeated these experiments, injecting suspensions of the pituitary glands of *Hoplius malabaricus* into two species of *Astyanax.* Mating activity was stimulated with an increase in size of both ovary and eggs over the untreated controls. When injections were made into *Prochilodus* of both sexes eggs and spermatozoa were obtained. Pituitary-like substances such as prolan and extracts of the pituitary gland have likewise been used in fishes with some success. Calvet (1932) placed young *Petromyzon* in aquaria containing pregnancy urine and found that the ovary increased markedly in size over that of the controls. Damas (1933) injected 30 lampreys with pregnancy urine and obtained expulsion of eggs in all cases. He also employed extracts of the pars anterior of the pituitary gland with similar results. Boucher,

Boucher and Fontaine (1934) injected the urine of pregnancy into eels and reported enlargement of the gonads in both sexes and evidence of the maturation of spermatozoa in the testis. On the other hand, wholly negative results were obtained by Koch and Scheuring (1936) after injecting *Phoxinus* with both prolan and an extract of the anterior lobe of the pituitary gland.

Effect of Injection of Pituitary Extracts

In an effort to determine whether or not the pituitary gland of *Fundulus heteroclitus* is concerned in the control of the sex cycle two methods were employed, the injection of pituitary glands or extracts of them, and extirpation of the pituitary body. The results of the injection method have been disappointing. In male individuals no effect which can be ascribed to the material injected has been observed. The following is a brief summary of the results of several experiments. Following the successful use by Burns and Buyse (1933) of extracts of mammalian pituitary glands in stimulating precocious sexual development in *Amblystoma,* injections of a crude, alkaline extract of the whole pituitary gland of sheep were made into the body cavity of *Fundulus.*[1] In the first series records were obtained from 28 males so injected, with a number of uninjected controls. While spermatozoa were obtained in a number of cases they were also obtained in control animals, developing in both groups after the individuals had been maintained in the laboratory for some time. This appearance of spermatozoa was possibly a result of the change from the lower temperature of the Delaware river in which they were collected to the higher temperature of the laboratory. At any rate, that the appearance of spermatozoa in these males was not a result of the injections was shown by further experiments. Twenty-four males were divided, 12 placed in an aquarium in a constant temperature room at 5° C. and the other 12 kept in an aquarium in which the temperature of the water varied from 18° to 22° C. with an average temperature during the experiment of 20°. Since the constant temperature room was illuminated by daylight the light conditions in the two rooms were fairly comparable. Half the animals in each room were injected daily with 0.1 cc. of sheep pituitary extract. Records were obtained on 18 of these animals. In the warm room the testes were activated and spermatozoa appeared in both uninjected and injected animals in all cases after 7 days. In the cold room no spermatozoa appeared in either injected or in control animals. Microscopic examination of the testes showed that those of the injected animals were comparable to those of the uninjected controls, those of both groups in the

[1] I wish to express my thanks to Dr. Oliver Kamm of the Research Laboratories of Parke, Davis and Company for this material.

cold room showing less activity than was the case in the testes of animals from the warm room.

In females a more varied injection program was employed in an attempt to produce ripe eggs. The following materials were injected: whole pituitary extract of sheep, Antuitrin S (Parke-Davis), extracts of fish pituitary glands (*Fundulus* and *Mustelus*), and freshly ground pituitary glands of *Fundulus*. Records were obtained from 35 injected females. Only 4 of these, animals injected in March and early April, delivered ripe eggs. The earliest case of this occurred on March 16 after the animal had been in the laboratory for 11 days and had received 3 injections of 0.15 cc. each of sheep pituitary extract. The other 3 cases delivered eggs late in April (April 20–28) about one month before eggs were obtained from normal animals (May 27). While these 4 cases suggest the possibility that injection of pituitary extracts into *Fundulus* in early spring after the ovocytes have grown to a certain size may cause them to mature more rapidly than they normally do, certainly the number of cases is too small to be of much significance. In the 31 other cases no ripe eggs were obtained and microscopic examination of the ovaries showed no significant differences between them and those of control animals.

Effect of Hypophysectomy

The second method employed to study the influence of the pituitary gland on the gonad cycle in *Fundulus* was the removal of the gland. The operation was performed essentially in the manner previously described (Matthews, 1933) but with several modifications. A single, short mid-ventral incision was made in the branchiostegal membrane, reaching anteriorly as far as the base of the tongue. Through this incision the tongue was dissected free from the tissues of the floor of the mouth. The tip of the tongue was then pulled backwards and held at right angles to the ventral surface of the head with the left hand, its wedge shape serving to hold the wound open. After cutting through the mucous membrane in the roof of the mouth the parasphenoid bone was cut through on three sides, bent to one side of the median line to expose the hypophysis, and then replaced after the pituitary body had been removed. If the original incision in the branchiostegal membrane was of the proper length the tongue could be tucked through the incision and the free tip would hold it in place, thus closing the wound in the branchiostegal membrane without sutures. Healing in these cases was much more satisfactory than in the earlier operations.

This operation was carried out at two periods of the year. One group of animals was operated on in March and April, the other in October, November and December. The mortality, as usual, was high.

Of 176 operated animals weight records and fixed gonads were obtained from only 73,—33 males and 40 females. When the gonads from these animals were examined differences were noted between them and

TABLE I

Percentage of total body weight formed by the testis in hypophysectomized Fundulus as compared with normal and operated controls

Normal and Operated Controls			Hypophysectomized Animals		
Date Killed	Days Post-Operative	GW/BW (per cent)	Date Killed	Days Post-Operative	GW/BW (per cent)
NOR Oct. 10.		0.22	Oct. 19	10	0.21
Oct. 10.		0.31	Oct. 19	13	0.19
Oct. 10.		0.27	Oct. 26	13	0.22
			Oct. 24	18	0.26
			Oct. 26	20	0.19
			Oct. 28	21	0.25
HYC Nov. 17.	39	0.76	Nov. 7	32	0.21
			Nov. 27	48	0.37
HYC Dec. 3.	53	0.71	Dec. 3	51	0.33
HYC Dec. 3.	56	0.49	Dec. 17	66	0.27
			Dec. 24	73	0.43
HYC Mar. 12.	12	0.46	Mar. 7	5	0.46
			Apr. 20	5	1.05
			Mar. 11	9	0.77
HYC May 5.	206	1.20	May 4	10	1.48
HYC May 5.	206	2.01	May 6	12	0.81
			May 6	12	1.62
			May 6	13	0.43
			May 8	14	0.24
			May 10	16	0.38
			May 10	16	0.54
			May 12	18	0.29
			May 13	19	0.44
			May 7	22	0.67
			May 14	29	0.41
			May 16	31	0.21
HYC June 11.	48	1.26	June 11	48	0.30
NOR June 11.		1.96	June 11	49	0.28
			June 11	55	0.17
			June 11	55	0.08
			June 11	57	0.28
			June 11	57	0.17
			May 5	206	0.15

those of controls, particularly in the males. These changes were apparent in the weight of the testis relative to the total body weight, and in both gross and microscopic appearance.

Table I summarizes the way in which the testis weight changes after

hypophysectomy. As has been shown (Matthews, 1938) during the normal seasonal cycle the testis forms the smallest percentage of the body weight from September to December and the greatest in May and June. When the pituitary gland is removed in October there is no significant difference in the weight of the testes of experimental animals as compared with those of controls even after 73 days. In the one animal which survived until the next breeding season, however (206 days), the difference in the proportion of the body weight formed by the testis as compared with that of control animals was striking. Obviously the testis in this animal had not enlarged with the onset of the breeding season as did that of the control. In the series from which the pituitary gland was removed in March and April and the animals killed during the next breeding season in May when the testis normally reaches its greatest size, the same difference between the weight of the testes of control and of hypophysectomized animals was noted, though in these cases they had been hypophysectomized only 48 to 57 days previously. How soon after operation this difference in weight is noticeable cannot be accurately determined from this series due to an insufficient number of animals killed early in the experiment. It is clear, however, that there is no difference up to about 10–12 days after removing the hypophysis. After 13 or 14 days, however, the proportion of the total body weight formed by the testis is consistently and notably less than in control animals.

In gross appearance the differences between the testes of hypophysectomized and control animals are clear from Figs. 1 and 2. In place of the large whitish testis of the control animal that of the hypophysectomized individual is smaller and has the gray, translucent appearance of the testes of sexually inactive animals killed in late fall.

The microscopic appearance of the testes of these animals also shows differences between control and hypophysectomized individuals. Although the testes of animals operated on in October show no significant weight differences as compared with those of controls, the microscopic structure of these testes is different. The testes of control animals killed in October show a large number of primary spermatogonia, a number of secondary cells, including both secondary spermatogonia and primary and secondary spermatocytes which are multiplying to form cortical cysts, as well as a number of spermatids and a few spermatozoa (Fig. 3). In two animals killed during mid-October from which the pituitary glands had been removed 10 and 13 days previously the primary spermatogonia are as numerous as in the controls and are dividing in both cases as indicated by the presence of mitotic figures. The striking differences between these two testes and those of control animals

killed at the same time lie in the reduced number of cells of the second type and in the small number of mitotic figures in these as compared with those in the control testes (Fig. 4). In fact, the cysts, which are prominent in the control testes, are small and poorly defined in those from the hypophysectomized individuals. Moreover, spermatids and spermatozoa are extremely scanty. The testes of these two hypophysectomized animals present the picture of multiplication of spermatogonia without subsequent maturation of sperm cells. If this lack of maturation of spermatozoa is due to the lack of the pituitary gland, then it might be expected that such differences would be more marked in those hypophysectomized animals allowed to live on into the normal breeding season when maturation of sperm cells is most rapid. That such is the case is indicated in a number of such cases. Obviously the removal of the pituitary gland must precede the killing of the animal by a sufficient number of days for the effect on the testis to be noted. Thus in none of the animals hypophysectomized in March and April and killed from five to twenty-two days later are any differences observable in the testes of the operated and control animals. In fact, one of these animals killed on April 20, five days after removal of the pituitary gland, showed a definitely active testis (Fig. 5). The differences between this testis and that of one of the same series of operated animals killed on May 16 are well marked (Fig. 6). In the latter case the only cells present are primary spermatogonia and a few spermatids and spermatozoa. The differences between the two are such as might be explained by assuming that on April 15, when the pituitary glands were removed, rapid multiplication and maturation of the spermatogenic cells were well under way in preparation for the next breeding season. In the animal killed five days after removal of the hypophysis the lack of the gland had not as yet produced any effect on the testis. Twenty-six

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Explanation of Plate I

Fig. 1. Testis of control animal which had its pituitary gland exposed but not removed on October 10 and was killed May 5. × 1.4.

Fig. 2. Testis of animal hypophysectomized on April 15 and killed June 11. × 1.4.

Fig. 3. Cross-section of testis of normal animal killed October 10. × 194. *C*, cortical cyst containing spermatocytes; *PS*, primary spermatogonia; *S*, spermatids.

Fig. 4. Cross-section of testis of animal hypophysectomized October 9 and killed October 19. × 194. *PS*, primary spermatogonia; *M*, medulla of testis.

Fig. 5. Cross-section of testis of animal hypophysectomized April 15 and killed April 20. × 80. *C*, cortical cysts containing cells which are dividing rapidly; *SZ*, spermatozoa.

Fig. 6. Cross-section of testis of animal hypophysectomized April 15 and killed May 16. × 80. *PS*, primary spermatogonia; *SZ*, spermatozoa.

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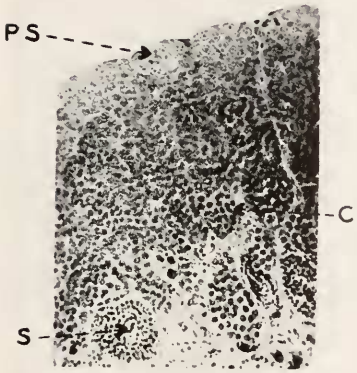

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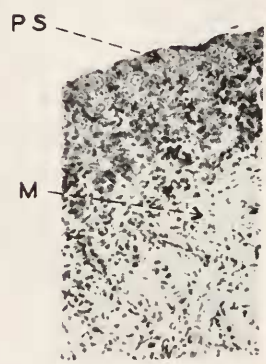

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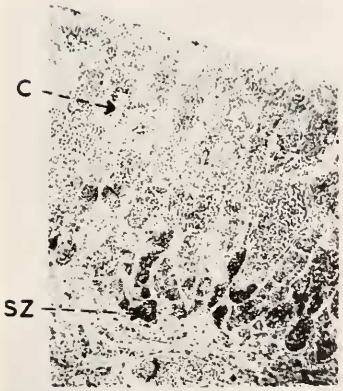

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PLATE I

days later, however, only a few spermatids and spermatozoa remain from the mass that had already been produced. Evidently after the lack of the pituitary gland has had time to manifest itself, further maturation of spermatogenic cells is either very much retarded or perhaps altogether lacking.

The most striking examples of this change in the testis following hypophysectomy were found in those animals which were allowed to run well over thirty days after removal of the pituitary gland and then killed close to or during the breeding season. Seven of these cases were observed with 5 control animals killed at the same time. These animals were killed from 48 to 206 days after removing the hypophysis.

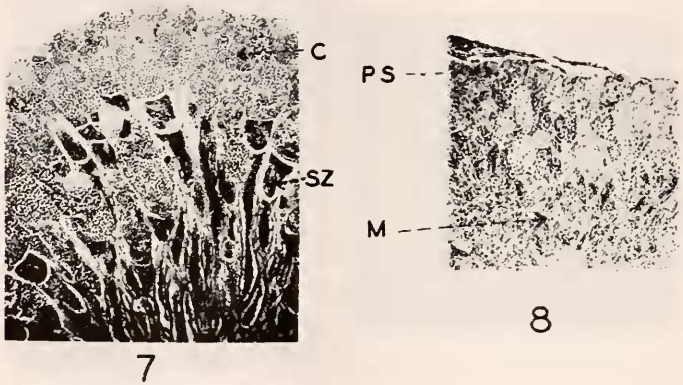

PLATE II

FIG. 7. Cross-section of testis of control animal which had its pituitary gland exposed but not removed on April 24 and was killed June 11. × 80. *C*, cortical cysts with cells which are dividing rapidly; *SZ*, spermatozoa.

FIG. 8. Cross-section of testis of animal hypophysectomized April 24 and killed June 11. × 80. *PS*, primary spermatogonia; *M*, medulla of testis.

With the exception of minor individual variations, differences in the microscopic appearance of the testes of these animals as compared with those of controls are much the same as those already described. The testis of all the hypophysectomized animals show only primary spermatogonia, a few scattered secondary spermatogonia and either very few spermatids and spermatozoa or none at all (Figs. 7 and 8). It might be noted, however, that in all these animals the primary spermatogonia are dividing, even in the one case from which the pituitary gland had been removed 206 days previously.

The data for hypophysectomized females are at present very scanty. As in the male, there is little change in weight of the female gonad in

those cases killed in the fall months and differences in the microscopic structure of the ovary of hypophysectomized as compared with control animals are hard to detect. Although more females were operated on than males the mortality of these animals was particularly high as the breeding season approached. As a result records on hypophysectomized females killed during May and June have been obtained on only a few animals. The oldest of these in post-operative age was killed only 55 days after removal of the hypophysis and records were obtained on only two others which were killed more than 30 days following hypophysectomy. In all three cases, however, the ovary was lighter relative to the total body weight than in control animals, several of which ran 206 days after exposing but not removing the pituitary gland. An example is provided in case *HY* 4/17–6/11 in which the ovary formed 1.52 per cent of the body weight 55 days after removing the hypophysis as compared with *HYC* 10/10–5/5 (206 days post-operative, 4 individuals) in which the average weight of the ovary constituted 2.87 per cent of the body weight. It is obvious, however, that more cases from which the hypophysis has been removed for more than 30 days prior to the normal breeding season must be obtained before the effect of the loss of the pituitary gland on the ovary may be accurately determined.

Summary

Injections of pituitary extracts into male *Fundulus* have been practically without stimulating effect on the testis. In females records were obtained from thirty-five cases of which only four delivered eggs earlier than did control animals, one case delivering ripe eggs six weeks, the other three cases about four weeks before the normal breeding season.

After removing the pituitary gland the gonads undergo regressive changes as compared with the controls, changes which are particularly striking in the testis. When the pituitary gland was removed in the fall and the animals were allowed to run until the next breeding season in May and June the testes failed to enlarge as they normally do. Microscopically such testes showed numerous primary spermatogonia but very few spermatocytes and practically no spermatozoa. When the pituitary gland was removed in March and April, after the testis had already enlarged somewhat, fourteen to twenty-one days after operation the testes had become notably smaller than in control animals, and showed only primary spermatogonia with a few spermatids and spermatozoa. These results indicate that the pituitary gland exerts a controlling influence on the seasonal cycle which the testis of this teleost fish exhibits. This influence is apparently of greater importance in maturation than in proliferation of the germ cells.

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